Kam[illegible]

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FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 14:1[illegible] ON [illegible] MAY [illegible]
L[illegible] [illegible] DUP REM L12 ([illegible] DUPLICATES REMOVED)
L2[illegible] 14 S L21 NOT L[illegible]

=> d que l[illegible]
L1 [illegible] SEA TSIEN R? AU
L2 [illegible] SEA CAMPBELL R?/AU
L3 [illegible] SEA L1 OR L2
L4 [illegible]4 SEA TANDEM(5A) FLUORESCENT (4A) PROTEIN#
L5 [illegible] SEA L4 AND L3
L6 [illegible] SEA DIMER? (5A) FLUORESCENT (4A) PROTEIN#
L7 15 SEA L6 AND L3
L10 [illegible] SEA (FUSION OR FUSED OR CHIMERA? OR CHIMAERA?) (5A) FLUORESCENT (4A) PROTEIN#
L11 [illegible] SEA L10 AND L3
L12 [illegible] SEA (L5 OR L7 OR L11) AND (GREEN OR GFP)
L22 14 DUP REM L12 (1[illegible] DUPLICATES REMOVED)

=> d ibib abs l22 1-14

L22 ANSWER 1 OF 14 HCAPLUS COPYRIGHT 2004 ACS
ACCESSION NUMBER: 2003:241[illegible] HCAPLUS
DOCUMENT NUMBER: 138:267491
TITLE: Monomeric and **dimeric** DsRed **fluorescent protein** variants and cDNAs and methods for preparing and using the proteins
INVENTOR(S): **Tsien, Roger Y.; Campbell, Robert E.**
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 67 pp., Cont.-in-part of U.S. Pat. Appl. 2003 32,088.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003 59835	A1	20030327	US 2002-121258	2002041[illegible]
US 2003 32088	A1	20030213	US 2001-866538	20010524

PRIORITY APPLN. INFO.: US [illegible] A2 [illegible]
US 2001-866538 A2 20010524

AB The present invention relates to monomeric and **dimeric** forms of Anthozoan **fluorescent proteins**, esp. Discosoma DsRed. The invention also relates to methods of making such **fluorescent protein** monomers and **dimers** using, for example, error-prone PCR. The improved fluorescent proteins may be used for assaying transcription as well as for anal. of in vivo localization or trafficking of proteins. Thus, DsRed variants which no longer tetramerize but instead form dimers, or exist as monomers were produced. The monomeric variant mRFP1 matures rapidly and has minimal emission when excited at wavelengths optimal for **GFP**. Application of knowledge gained with the DsRed system to mutagenesis of **GFP** resulted in **GFP** mutants which no longer dimerized.

Search completed by David Schreiber [illegible]-4292

[illegible]

L22 ANSWER [illegible] OF 1[illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: 2[illegible] HCAPLUS
DOCUMENT NUMBER: 1[illegible]
TITLE: **Dimeric fusion** [illegible] **fluorescent proteins** showing low levels of oligomerization
INVENTOR(S): **Tsien, Roger Y.; Campbell, Robert E.**
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. [illegible], [illegible] pp., [illegible]-in-part of U.S. Ser. No. [illegible]
CODEN: [illegible]
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

7 [illegible]

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	A1	[illegible]	[illegible]	[illegible]
W[illegible]	A2	[illegible]	W[illegible]	[illegible]
W[illegible]	A[illegible]	[illegible]		
W: AE, A[illegible], AL, AM, AT, AU, AZ, BA, BB, B[illegible], BR, BY, BZ, CA, CH, C[illegible], [illegible] GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, [illegible] RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003059835	A1	20030327	US 2002-121258	20020410

PRIORITY APPLN. INFO.: US 2001-794306 A2 20010226
US 2001-866538 A 20010524

AB Fluorescent proteins, such as **green** fluorescent protein, that show lower degrees of oligomerization are described for anal. use. The non-oligomerizing derivs. are **fusion proteins** of **fluorescent proteins** including a naturally occurring **green** fluorescent **protein**, a red **fluorescent protein** or derivs. that are **dimers** of the **fluorescent protein** connected by a linker peptide. The protein may also carry other modifications, such as in surface residues, that prevent oligomerization without a significant impact on fluorescence properties. Also provided is a fusion protein, which includes a non-oligomerizing fluorescent protein linked to at least one polypeptide of interest. Chimeric genes encoding these fusion proteins and expression constructs and hosts for the genes are also described.

L22 ANSWER [illegible] OF 1[illegible] HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:[illegible] HCAPLUS
DOCUMENT NUMBER: 137:[illegible]
TITLE: Non-oligomerizing fluorescent proteins and their uses
INVENTOR(S): **Tsien, Roger Y.**; Baird, Geoffrey S.; **Campbell, Robert E.**; Zacharias, David A.
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., [illegible] pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

Search completed by David Schreiber [illegible]

FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	A2	[illegible]	[illegible]	[illegible]
[illegible]	A3	[illegible]		

W: [illegible]

RW: [illegible]

US [illegible]	A1	[illegible]	US 2001-[illegible]	[illegible]

PRIORITY APPLN. INFO.: US [illegible] A [illegible]
US [illegible] A [illegible]

AB Non-oligomerizing fluorescent proteins, which are formed by operatively linking two or more monomers of a fluorescent protein, or which are derived from a fluorescent protein having at least one mutation that reduces or eliminates the ability of the fluorescent protein to oligomerize, are provided. The non-oligomerizing fluorescent proteins can be derived from a naturally occurring **green** fluorescent protein, a red fluorescent protein, or other fluorescent protein, or a fluorescent protein related thereto. Also provided is a fusion protein, which includes a non-oligomerizing fluorescent protein linked to at least one polypeptide of interest. In addn., a polynucleotide encoding a non-oligomerizing fluorescent protein is provided, as is a recombinant nucleic acid mol., which includes polynucleotide encoding a non-oligomerizing fluorescent protein operatively linked to at least a second polynucleotide. Vectors and host cells contg. such polynucleotides also are provided, as are kits contg. one or more non-oligomerizing fluorescent proteins or encoding polynucleotides or constructs derived therefrom. Further provided are methods of making and using the proteins and polynucleotides, e.g., to det. pH in cells or biol. tissues, to det. the presence or activity of enzymes, to identify agents or conditions which regulate the activity of an expression control sequence, and to identify interactions of a first and second mol. Thus, residues 206-alanine, 221-leucine, and 223-phenylalanine were shown to be involved in oligomerization of **GFP**. Substitution of charged amino acids (e.g., lysine, arginine) for these residues prevented oligomerization.

L22 ANSWER 4 OF 1[illegible] MEDLINE DUPLICATE 1
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: A monomeric red fluorescent protein.
AUTHOR: **Campbell Robert E**; Tour [illegible]; Palmer Amy E; Steinbach Paul A; Baird Geoffrey S; Zacharias David A; **Tsien Roger Y**
CORPORATE SOURCE: Department of Pharmacology, University of California at San Diego, [illegible] Gilman Drive, La Jolla, CA [illegible], USA.
CONTRACT NUMBER: [illegible] (NIH)
[illegible] (NIGMS)
[illegible] (NINDS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, [illegible]

Search completed by [illegible] Schreiber [illegible]

Nam [illegible]

[illegible] ISSN: [illegible]
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF [illegible]; GENBANK-AF [illegible]; GENBANK-AF [illegible]
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB All [illegible] fluorescent proteins [illegible] display [illegible] quaternary structure, including the weak tendency of Aequorea **green fluorescent protein** (**GFP**) to **dimerize**, the [illegible] dimerization of Renilla **GFP**, and the obligate tetramerization of the red fluorescent protein from [illegible] (DsRed). Although the weak dimerization of Aequorea **GFP** has not impeded its acceptance as an indispensable tool of cell biology, the obligate tetramerization of DsRed has greatly hindered its use as a genetically encoded fusion tag. We present here the stepwise evolution of DsRed to a dimer and then either to a genetic fusion of two copies of the protein, i.e., a tandem dimer, or to a true monomer designated mRFP1 (monomeric red fluorescent protein). Each subunit interface was disrupted by insertion of arginines, which initially crippled the resulting protein, but red fluorescence could be rescued by random and directed mutagenesis totaling [illegible] substitutions in the dimer and [illegible] in mRFP1. Fusions of the gap junction protein connexin43 to mRFP1 formed fully functional junctions, whereas analogous fusions to the tetramer and dimer failed. Although mRFP1 has somewhat lower extinction coefficient, quantum yield, and photostability than DsRed, mRFP1 matures [illegible] times faster, so that it shows similar brightness in living cells. In addition, the excitation and emission peaks of mRFP1, 584 and 6[illegible] nm, are approximately 25 nm red-shifted from DsRed, which should confer greater tissue penetration and spectral separation from autofluorescence and other fluorescent proteins.

L21 ANSWER 5 OF 1[illegible] MEDLINE DUPLICATE 2
ACCESSION NUMBER: [illegible]2249561 MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: 11988576
TITLE: Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells.
COMMENT: Comment in: Science. 2002 May 3;296(5569):855-7
AUTHOR: Zacharias David A; Violin Jonathan D; Newton Alexandra C; **Tsien Roger Y**
CORPORATE SOURCE: Department of Pharmacology, Biomedical Sciences Graduate Program, and, Howard Hughes Medical Institute, University of California, San Diego, La Jolla, CA 92093-0647, USA.
CONTRACT NUMBER: [illegible] GM07752 (NIGMS)
DK[illegible]4441 (NIDDK)
[illegible] (NINDS)
SOURCE: SCIENCE, (2002 May 3) 296 (5569) 913-6.
Journal code: [illegible]. ISSN: [illegible]
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB Many proteins associated with the plasma membrane are known to partition

Search completed by David Schreiber [illegible]-4291

into dynamic clusters of sphingolipid- and cholesterol-rich domains called lipid rafts, but the determinants dictating this segregation of proteins in the membrane are poorly understood. We suppressed the tendency of Aequorea **fluorescent proteins** to **dimerize** and targeted these variants to the plasma membrane using several different types of lipid anchors. Fluorescence resonance energy transfer measurements in living cells revealed that acyl but not prenyl modifications promote clustering in lipid rafts. Thus the nature of the lipid anchor on a protein is sufficient to determine submicroscopic localization within the plasma membrane.

L22 ANSWER [illegible] OF [illegible] BIOSIS COPYRIGHT [illegible] BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: [illegible] BIOSIS
DOCUMENT NUMBER: PREV[illegible]
TITLE: A distinct bipartite motif is required for the localization of inhibitory kappaB-like (IkappaBL) protein to nuclear speckles.
AUTHOR(S): Semple, Jennifer I.; Brown, Stephanie E.; Sanderson, Christopher M.; **Campbell, R. Duncan (1)**
CORPORATE SOURCE: (1) Functional Genomics Group, MRC UK HGMP Research Centre, Hinxton, Cambridge, CB10 1SB: rcampbel@hgmp.mrc.ac.uk UK
SOURCE: Biochemical Journal, [illegible] February, [illegible] Vol. [illegible], No. [illegible], pp. 4[illegible]-4[illegible]. http://www.biochemj.org/. print. ISSN: [illegible].
DOCUMENT TYPE: Article
LANGUAGE: English
AB The inhibitory kappaB (IkappaB)-like (IkappaBL) gene is located within the Class III region of the MHC on human chromosome 6. Previous analysis of the predicted amino acid sequence of the human IkappaBL protein revealed three putative functional domains; two ankyrin repeat sequences, which are similar to the second and third ankyrin repeats of the nuclear factor kappaB (NF-kappaB) protein; three PEST sequence motifs (a sequence that is rich in proline, serine, aspartic acid and threonine residues), which are also found in other IkappaB family members; and a C-terminal leucine zipper-like motif. In the present study we have identified a novel bipartite motif, which is required for nuclear localization of the IkappaBL protein. Analyses of IkappaBL-specific transcripts revealed the existence of a widely expressed spliced variant form of IkappaBL (IkappaBLsv1), which lacks the amino acid sequence SELEDEWQEYNGRFR (where single-letter amino-acid notation has been used). Interestingly, translation of IkappaBL mRNA in vivo was found to initiate predominantly from the second available methionine, thereby resulting in the disruption of the predicted N-terminal PEST sequence. Also, transient expression of T7 epitope-tagged IkappaBL and IkappaBLsv1 proteins in mammalian cells showed that both proteins were targeted to the nucleus, where they accumulate in nuclear speckles. To define the protein domains required for nuclear import and subnuclear localization, a complementary set of deletion mutants and enhanced **green** fluorescent protein-IkappaBL domain fusions were expressed in mammalian cells. Data from these experiments show that a combination of the ankyrin-repeat region and an adjacent arginine-rich sequence are necessary and sufficient for both nuclear import and speckle localization.

L22 ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: Modified **green** fluorescent proteins of Aequorea
INVENTOR(S): **Tsien, Roger Y.**; Heim, Roger

Man [illegible]

PATENT ASSIGNEE(S): Regents of the University of California, USA
SOURCE: U.S., [illegible] pp., Cont.-in-part of U.S. [illegible].
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US [illegible]	B1	[illegible]	US [illegible]	[illegible]
US 5625048	A	19970429	US 1994-337915	19941110
CA 2205[illegible]	AA	19960523	CA 1995-2205066	19951113
WO 9623810	A1	19960523	WO 1995-US14692	19951113
W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
EP 11[illegible]	A2	20010606	EP 2001-105[illegible]	19951113
EP 11[illegible]	A3	20020918		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
US [illegible]	A	19980707	US 1996-753145	19961120

PRIORITY APPLN. INFO.:
US 1994-337915 A2 19941110
WO 1995-US14692 W 19951113
CA 1995-2205066 A3 19951113
EP 1995-939898 A3 19951113
US 1996-727452 A1 19961016

AB Modifications in the sequence of Aequorea wild-type **GFP** provide products having markedly different excitation and emission spectra from corresponding products from wild-type **GFP**. In one class of modifications, the product derived from the modified **GFP** exhibits an alteration in the ratio of two main excitation peaks obsd. with the product derived from wild-type **GFP**. In another class, the product derived from the modified **GFP** fluoresces at a shorter wavelength than the corresponding product from wild-type **GFP**. In yet another class of modifications, the product derived from the modified **GFP** exhibits only a single excitation peak and enhanced emission relative to the product derived from wild-type **GFP**.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER [illegible] OF 19 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001429679 MEDLINE
DOCUMENT NUMBER: 21369963 PubMed ID: 11487331
TITLE: Reducing the environmental sensitivity of yellow fluorescent protein. Mechanism and applications.
AUTHOR: Griesbeck O; Baird G S; **Campbell R E**; Zacharias D A; **Tsien R Y**
CORPORATE SOURCE: Howard Hughes Medical Institute, University of California, San Diego, La Jolla, California 92093-0647, USA.
CONTRACT NUMBER: [illegible]CA23100-16 (NCI)
NS-[illegible] (NINDS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Aug [illegible]) [illegible] 29188-94.
Journal code: 2985121R. ISSN: 0021-9258.

Search completed by David Schreiber [illegible]-4292

[illegible]

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: [illegible]
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB Yellow mutants of the **green** fluorescent protein (YFP) are crucial constituents of genetically encoded indicators of signal transduction and fusions to monitor protein-protein interactions. However, previous YFPs show excessive pH sensitivity, chloride interference, poor photostability, or poor expression at 37 degrees C. Protein evolution in Escherichia coli has produced a new YFP named Citrine, in which the mutation Q69M confers a much lower pKa ([illegible]) than for previous YFPs, indifference to chloride, twice the photostability of previous YFPs, and much better expression at 37 degrees C and in organelles. The halide resistance is explained by a [illegible]-A x-ray crystal structure of Citrine, showing that the methionine side chain fills what was once a large halide-binding cavity adjacent to the chromophore. Insertion of calmodulin within Citrine or **fusion** of cyan **fluorescent protein**, calmodulin, a calmodulin-binding peptide and Citrine has generated improved calcium indicators. These chimeras can be targeted to multiple cellular locations and have permitted the first single-cell imaging of free [Ca2+] in the Golgi. Citrine is superior to all previous YFPs except when pH or halide sensitivity is desired and is particularly advantageous within genetically encoded fluorescent indicators of physiological signals.

L2[illegible] ANSWER [illegible] OF 1[illegible] HCAPLUS COPYRIGHT 20[illegible] ACS
ACCESSION NUMBER: 2001:657326 HCAPLUS
DOCUMENT NUMBER: 136:321466
TITLE: Fluorescence resonance energy transfer analysis of cell surface receptor interactions and signaling using spectral variants of the **green** fluorescent protein
AUTHOR(S): Chan, Francis Ka-Ming; Siegel, Richard M.; Zacharias, David; Swofford, Ruth; Holmes, Kevin L.; **Tsien, Roger Y.**; Lenardo, Michael J.
CORPORATE SOURCE: Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892-1892, USA
SOURCE: Cytometry (2001), 44(4), 361-368
CODEN: CYTODQ; ISSN: 0196-4763
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Fluorescence resonance energy transfer (FRET) is a powerful technique for measuring mol. interactions at Angstrom distances. We present a new method for FRET that utilizes the unique spectral properties of variants of the **green** fluorescent protein (**GFP**) for large-scale anal. by flow cytometry. The proteins of interest are fused in frame resp. to the cyan fluorescent protein (CFP) or the yellow fluorescent protein (YFP). FRET between these differentially tagged fusion proteins is analyzed using a dual-laser FACSVantage cytometer. We show that homotypic interactions between individual receptor chains of tumor necrosis factor receptor (TNFR) family members can be detected as FRET from CFP-tagged receptor chains to YFP-tagged receptor chains. Noncovalent mol.

[illegible] can be detected as FRET between fusions of CFP and YFP to either the intracellular or extracellular regions of the receptor chains. The specificity of the assay is demonstrated by the absence of FRET between proteins from receptor pairs that do not [illegible] with each other. Interaction between a TNFR-like receptor [illegible] and a [illegible] signaling component [illegible] can also be demonstrated by flow cytometric FRET anal. The ability of spectral variants of **GFP** to allow cytometric FRET anal. of membrane receptors is demonstrated. This method of analyzing FRET allows probing of [illegible] interactions that involve both the intracellular and extracellular regions of membrane proteins as well as proteins within the cells. [illegible] flow cytometry, FRET allows the quant. detn. of [illegible] mol. [illegible] at [illegible] level in living cells. Moreover, flow cytometry allows quant. analyses to be carried out on a cell-by-cell basis on large no. of cells.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS

ACCESSION NUMBER: [illegible] HCAPLUS

DOCUMENT NUMBER: [illegible]

TITLE: **Green** fluorescent protein analogs containing ligand-binding domain peptides for use as reporter molecules

INVENTOR(S): **Tsien, Roger Y.**; Baird, Geoffrey A.

PATENT ASSIGNEE(S): Regents of the University of California, USA

SOURCE: PCT Int. Appl., [illegible] pp. CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2[illegible]	A2	2[illegible]	WO 2000-US13684	2000051[illegible]
WO 2[illegible]	C2	200[illegible]		

W: AE, A[illegible], AL, AM, AT, A[illegible], AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, [illegible], CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, [illegible], IL, IN, IS, [illegible], KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	A1	[illegible]	[illegible]	[illegible]
AU [illegible]	A5	2[illegible]	AU [illegible]	[illegible]
[illegible]	A1	2[illegible]	[illegible]	[illegible]

PRIORITY APPLN. INFO.:

APPLICATION NO.	KIND	DATE
US 1999-316919	A	1999[illegible]21
US 1999-316920	A	1999[illegible]21
WO 2[illegible]-US13684	W	[illegible]

AB The present invention provides polypeptide and polynucleotides encoding fluorescent indicators having inserted within a fluorescent moiety a [illegible] polypeptide. The proteins are [illegible] that are not normally fluorescent as a result of FRET coupling. [illegible] binding of a ligand to the [illegible] results in a conformational change and an increase in the [illegible] of the protein. Also provided are methods of using the fluorescent indicators. [illegible] permuted fluorescent polypeptides and

Fax [illegible], [illegible]

[illegible]

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: Fluorescent protein sensors for measuring the pH of a biological sample
INVENTOR(S): **Tsien, Roger Y.**; [illegible]; [illegible], Rebekka M.; Remington, S. James
PATENT ASSIGNEE(S): The Regents of the University of California, USA; The State of Oregon Acting by and Through the State Board of Higher Educ.
SOURCE: [illegible], [illegible] pp., Cont.-in-part of U.S. Ser. No. [illegible]
CODEN: [illegible]
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	A	[illegible]	[illegible]	[illegible]
[illegible]	A	[illegible]	[illegible]	[illegible]
W[illegible]	A2	[illegible]	W[illegible]	[illegible]
W[illegible]	A[illegible]	[illegible]		

W: [illegible]
RW: AT, [illegible], [illegible], [illegible], DE, DK, ES, FI, FR, [illegible], [illegible], IE, IT, LU, [illegible], NL, [illegible], [illegible]

PRIORITY APPLN. INFO.: [illegible] A[illegible] [illegible]
[illegible] 1998-172063 A [illegible]

AB Disclosed are fluorescent protein sensors for measuring the pH of a sample, nucleic acids encoding them, and methods of use. The preferred fluorescent protein sensors are variants of the **green** fluorescent protein (**GFP**) from Aequorea victoria. Also disclosed are compns. and methods for measuring the pH of a specific region of a cell, such as the mitochondrial matrix or the Golgi lumen. Enhanced **green** and enhanced yellow fluorescent proteins (recombinantly prepd.) were used as indicators of pH in the cytosol or nucleus. To construct proteins to use as pH indicators in the Golgi app., polynucleotides encoding the [illegible]-N-terminal amino acids of the type II membrane-anchored protein galactosyltransferase were ligated to polynucleotides encoding the mutant **green** fluorescent proteins.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER 1[illegible] OF 19 HCAPLUS COPYRIGHT 200[illegible] ACS
ACCESSION NUMBER: [illegible]:140117 HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: A genetically encoded, fluorescent indicator for cyclic AMP in living cells
AUTHOR(S): Zaccolo, Manuela; De Giorgi, Francesca; Cho, Charles Y.; Feng, Luxin; Knapp, Tim; Negulescu, Paul A.; Taylor, Susan S.; **Tsien, Roger Y.**; Pozzan, Tullio
CORPORATE SOURCE: Department of Experimental Biomedical Sciences, University of Padova, Padua, [illegible], Italy
SOURCE: Nature Cell Biology (200[illegible]), [illegible], [illegible]
CODEN: NCBIFN; ISSN: 14[illegible]
PUBLISHER: Macmillan Magazines Ltd

[illegible]

DOCUMENT TYPE: [illegible]
LANGUAGE: English
AB [illegible] several signaling [illegible] within cells, and changes in the [illegible] of this second messenger have an essential role in many cellular [illegible]. Here we describe a new method for monitoring the [illegible] of cAMP in living cells. By tagging the cAMP effector protein kinase A with two suitable **green** fluorescent protein mutants, we have [illegible] in which the fluorescence resonance energy transfer between the two fluorescent [illegible] is dependent on the levels of cAMP. [illegible] cAMP [illegible].
REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] BIOSIS COPYRIGHT [illegible] BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: [illegible] BIOSIS
DOCUMENT NUMBER: PREV[illegible]
TITLE: Monitoring exocytosis by quenching of **green** fluorescent protein fluorescence in the lumen of synaptic vesicles.
AUTHOR(S): [illegible]; Harata, N.; [illegible]; **Tsien, R. W.**
CORPORATE SOURCE: [illegible] Stanford Univ., Stanford, CA USA
SOURCE: Society for Neuroscience Abstracts, [illegible] Vol. 26, No. [illegible], pp. Abstract No.-[illegible]. print. Meeting Info.: [illegible] Annual Meeting of the Society for Neuroscience New Orleans, LA, USA November [illegible] Society for Neuroscience . ISSN: [illegible].
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English
AB FM dyes allow fluorescence imaging of active presynaptic terminals and provide valuable insights into dynamics of vesicular turnover, but are not ideally suited for studying the kinetics of exocytosis. FM1-43, for example, requires approx. [illegible] s to departition from the membrane. To selectively monitor exocytosis independent of endocytosis we have developed a new approach based on the quenching of a fluorophore permanently held within the vesicle lumen by a probe that enters vesicles upon **fusion**. The fluorophore is **green fluorescent protein** (**GFP**) associated with the lumenal domain of VAMP (synaptobrevin-2), introduced by transfection, and the quencher is bromophenol blue (BPB), a small, hydrophilic molecule. In quenching experiments, boutons were first exposed to BPB in Ca2+-free Tyrode to eliminate fluorescence of any VAMP-**GFP** that had somehow been stranded on the plasma membrane. Subsequent exposure to [illegible] mM K+/2 mM Ca2+ to induce voltage-gated Ca2+ entry caused sharp drop in **GFP** fluorescence (n=7), with biphasic kinetics reminiscent of destaining of FM1-43. In contrast, high K+/Ca2+ stimulation in the absence of BPB failed to decrease the fluorescence of puncta, even though a later repeat of the same challenge in BPB caused clear decreases in fluorescence in the same puncta (n=[illegible]). Evidently, pH increases during exocytosis had no significant effect on the fluorescence of this **GFP** construct. When BPB-loaded boutons were subjected to high K+/Ca2+ stimulation in the absence of BPB, the fluorescence of puncta suddenly increased (n=[illegible]), as expected if BPB had escaped from vesicles undergoing fusion. One advantage of this approach is that VAMP-**GFP** can mark boutons even if they are functionally silent. The exocytosis of quencher-loaded vesicles causes a fluorescence increase, advantageous for picking out signals against [illegible]. Extending this approach to quenchers of different size

[illegible]

[illegible]

L[illegible] ANSWER [illegible] OF 1[illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: **Green** fluorescent proteins for measuring intracellular pH in a biological sample
INVENTOR(S): **Tsien, Roger Y.**; [illegible]
PATENT ASSIGNEE(S): The Regents of the University of California, USA; University of [illegible]
SOURCE: PCT Int. Appl., [illegible]
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	A[illegible]	[illegible]	W[illegible]	[illegible]
[illegible]	A[illegible]	[illegible]		
W: [illegible]				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
[illegible]	A	[illegible]	[illegible]	[illegible]
[illegible]	A	[illegible]	[illegible]	[illegible]

PRIORITY APPLN. INFO.: [illegible] A [illegible]
[illegible] A [illegible]

AB [illegible] are **green** fluorescent protein sensors, including their protein and encoding nucleotide sequences, which show reversible fluorescence changes of physiol. pH ranges for measuring the pH of a sample, and methods of use for measuring the pH of specific intracellular regions. The preferred fluorescent protein sensors are variants of the **green** fluorescent protein (**GFP**) from Aequora victoria. Also disclosed are compns. and methods for measuring the pH of a specific region of a cell, such as the mitochondrial matrix or the Golgi lumen. Some sequence listings were not provided by the authors.

L[illegible] ANSWER [illegible] OF 1[illegible] HCAPLUS COPYRIGHT 2[illegible] ACS
ACCESSION NUMBER: 1999:[illegible] HCAPLUS
DOCUMENT NUMBER: 131:34[illegible]
TITLE: **Tandem fluorescent protein** constructs and their preparation for enzyme assays
INVENTOR(S): **Tsien, Roger Y.**; Heim, Roger; Cubitt, Andrew
PATENT ASSIGNEE(S): The Regents of the University of California, USA; Aurora Biosciences Corporation
SOURCE: U.S., 33 pp., Cont.-in-part of U.S. Ser. No. [illegible]
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
[illegible]	A	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]
PRIORITY APPLN. INFO.: [illegible]

AB [illegible] tandem fluorescent protein [illegible] including a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties. The donor and acceptor moieties exhibit fluorescence resonance energy transfer which is eliminated upon cleavage. The constructs are useful in enzymatic assays. Mutant green fluorescent proteins (GFP) were [illegible] of the Aequorea victoria GFP. Polyhistidine-tagged tandem green [illegible] fluorescent proteins were recombinantly constructed having an inserted peptide sequence including cleavage [illegible] sites for many proteases. Cleavage expts. were done with trypsin, enterokinase and calpain.

REFERENCE COUNT: [illegible] THERE ARE [illegible] CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] MEDLINE [illegible]
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Circular permutation and receptor insertion within green fluorescent proteins.
AUTHOR: Baird G S; Zacharias D A; Tsien R Y
CORPORATE SOURCE: Department of Pharmacology, University of California at San Diego, La Jolla, CA [illegible], USA.
CONTRACT NUMBER: NS[illegible] (NINDS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Sep [illegible]) [illegible] 11241-[illegible].
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 1999[illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB Many areas of biology and biotechnology have been revolutionized by the ability to label proteins genetically by fusion to the Aequorea green fluorescent protein (GFP). In previous fusions, the GFP has been treated as an indivisible entity, usually appended to the amino or carboxyl terminus of the host protein, occasionally inserted within the host sequence. The tightly interwoven, three-dimensional structure and intricate posttranslational self-modification required for chromophore formation would suggest that major rearrangements or insertions within GFP would prevent fluorescence. However, we now show that several rearrangements of GFPs, in which the amino and carboxyl portions are interchanged and rejoined with a short spacer connecting the original termini, still become fluorescent. These circular permutations have altered pKa values and orientations of the chromophore with respect to a fusion partner. Furthermore, certain locations within GFP tolerate insertion of entire proteins, and conformational changes in the insert can have profound effects on the fluorescence. For example, insertions of calmodulin or a zinc finger domain in place of Tyr-145 of a yellow mutant (enhanced yellow fluorescent protein) of GFP result in indicator proteins whose fluorescence can be enhanced

Search completed by David Schreiber [illegible]

[illegible] calmodulin [illegible] enhanced yellow fluorescent protein [illegible] mammalian cells. [illegible] GFP [illegible] a new strategy for [illegible] physiological indicators.

L12 ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: Fluorescent protein sensors [illegible] green fluorescent protein and calmodulin for the detection of analytes
INVENTOR(S): **Tsien, Roger Y.**; Miyawaki, Atsushi
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., [illegible] pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO [illegible]	A1	[illegible]	WO 1998-US4978	[illegible]
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US [illegible]	A	[illegible]	US 1997-818253	19970314
US [illegible]	B1	20010306	US 1997-818252	19970314
AU [illegible]	A1	[illegible]	AU 1998-67023	19980313
EP [illegible]	A1	[illegible]	EP 1998-[illegible]	19980313
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US [illegible]	A1	[illegible]	US [illegible]	[illegible]

PRIORITY APPLN. INFO.:

US 1997-818252	A	19970314
US 1997-818253	A	19970314
US 1997-919143	A	19970827
WO 1998-US4978	W	19980313

AB Fluorescent indicators including a binding protein moiety, a donor fluorescent protein moiety, and an acceptor fluorescent protein moiety are described. The binding protein moiety has an analyte-binding region which binds an analyte and causes the indicator to change conformation upon exposure to the analyte. The donor moiety and the acceptor moiety change position relative to each other when the analyte binds to the analyte-binding region. The donor moiety and the acceptor moiety exhibit fluorescence resonance energy transfer when the donor moiety is excited and the distance between the donor moiety and the acceptor moiety is small. The indicators can be used to measure analyte concns. in samples, such as calcium ion concns. in cells. For example, a fluorescent indicator for Ca2+ was produced by sandwiching a Xenopus calmodulin-M13 fusion between a [illegible] Y66H Y145F and a **green** (S65T) mutant of Aequorea **green** fluorescent protein (**GFP**), where M13 is the calmodulin-binding region of myosin light-chain kinase. Chimeric proteins (cameleon-1) incorporating a polyhistidine tag were

[illegible]

[illegible] in Escherichia coli, purified by nickel-chelate and size-exclusion chromatography, and their fluorescence characterized. [illegible] rapidly changes emission [illegible] by retracting and extending a [illegible] of the calmodulin, the ratio of [illegible] nm to 440 nm emission increased by [illegible] upon binding [illegible]. Improved expression in mammalian cells is achieved by introducing mammalian [illegible] into the cDNA and mutating [illegible], and [illegible].

REFERENCE COUNT: [illegible] THERE ARE [illegible] CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS

ACCESSION NUMBER: 1998:[illegible] HCAPLUS
DOCUMENT NUMBER: 129:[illegible]
TITLE: Fluorogenic assay substrates for proteinases with cleavage sites flanked by moieties that exhibit fluorescence resonant energy transfer
INVENTOR(S): **Tsien, Roger Y.**; Heim, Roger; Cubitt, Andrew
PATENT ASSIGNEE(S): Regents of the University of California, USA; Aurora Biosciences Corp.
SOURCE: PCT Int. Appl., [illegible] pp. CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: [illegible]
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO [illegible]	A1	[illegible]	WO 1997-[illegible]	[illegible]
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU [illegible]	A1	[illegible]	AU 1997-22504	[illegible]
AU [illegible]	B2	[illegible]		
EP [illegible]	A1	19981118	EP 1997-905667	19970131
EP [illegible]	B1	[illegible]		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
AT [illegible]	E	[illegible]	AT 1997-905667	[illegible]
ES [illegible]	T3	[illegible]1216	ES 1997-905667	[illegible]

PRIORITY APPLN. INFO.: US 1996-594575 A [illegible]
WO 1997-[illegible] W [illegible]

AB Fluorogenic peptide assay substrates for proteinases are described. The peptides are derived from the cleavage site of the proteinase and are flanked by fluorescent donor and acceptor groups. The donor and acceptor moieties exhibit fluorescence resonance energy transfer (FRET) that is eliminated upon cleavage. The fluorescent groups may be added to the peptide chem. or the cleavage peptide may be part of a larger protein that exhibits natural fluorescence resonance energy transfer, e.g. a deriv. of **green** [illegible] protein. A pair of peptides exhibiting FRET [illegible] assay many cleavage reactions where the group of interest [illegible]. A **fusion protein** of a **green fluorescent protein** and a blue [illegible]

[illegible]

[illegible] protein [illegible] by a linker that includes cleavage sites for trypsin, [illegible] was [illegible] by expression [illegible]. The **fusion protein** [illegible] **green fluorescence.** When cleaved by trypsin, the **green** [illegible] disappears almost completely and [illegible]. The [illegible] **green** [illegible] fluorescent [illegible] changes in fluorescence indicating that they were resistant to trypsin.

[illegible]
[illegible]
TITLE: Fluorescent [illegible] of intracellular [illegible] transduction: Applications to pharmaceutical screening.
AUTHOR(S): **Tsien, R. Y.**; [illegible]; [illegible], A. B.; [illegible], [illegible]; [illegible], [illegible]. E.
CORPORATE SOURCE: Howard Hughes Med. Inst., UCSD, La Jolla, CA, [illegible], USA
SOURCE: [illegible] Abstracts, [illegible] ACS National Meeting, San Francisco, April 13-17, 1997, [illegible]-474. American Chemical Society: Washington, D. C.
CODEN: 64AOAA
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB [illegible] of intracellular signal transduction, [illegible] with [illegible] cell lines and miniaturized [illegible] assays, provide some promising approaches to ultra-high-throughput screening of pharmaceutical libraries. A) **Proteins** can be **fluorescently** tagged in vivo by **fusion** to **Green Fluorescent Protein** (**GFP**). Mutagenesis, [illegible] in part by the crystal structure, has improved **GFP** and yielded new colors, which reveal protein-protein interaction and protease activity by fluorescence resonance energy transfer (FRET). B) Gene expression can be visualized in single living cells by using .beta.-lactamase as a reporter enzyme. It cleaves novel membrane-permeant substrates and changes their fluorescence from **green** to blue by disrupting FRET. Because of its enzymic amplification and nondestructive assay, .beta.-lactamase has major advantages over existing reporter genes. C) A fast and sensitive optical readout of membrane voltage results from FRET between a fluorescent gating charge and another fluorophore attached to one side of the membrane.

=> d [illegible]
L1 [illegible] SEA TSIEN R?/AU
L2 [illegible] SEA CAMPBELL R?/AU
L3 [illegible] SEA L1 OR L2
L4 [illegible] SEA TANDEM(5A) [illegible] PROTEIN#
L5 [illegible] SEA L4 AND L3
L6 [illegible] SEA DIMER?(5A) FLUORESCENT(5A) PROTEIN#
L7 [illegible] SEA L6 AND L3
L8 [illegible] SEA COVALENT(5A) FLUORESCENT(5A) PROTEIN#
L9 [illegible] SEA (FUSION OR FUSED OR CHIMER? OR CHIMAER?) (5A) FLUORESCENT (5A) PROTEIN#
L11 [illegible] SEA L1? AND L?
L12 [illegible] SEA (L? OR L? OR L11) AND (GREEN OR GFP)
L13 [illegible] SEA (OLIGOMER?(5A) FLUORESCENT(5A) PROTEIN#
L14 [illegible] SEA (NONOLIGOMER? OR NON-OLIGOMER? OR NON OLIGOMER?) (5A) FLUORESCENT (5A) PROTEIN#

Search completed by David Schreiber [illegible]-4292

[illegible]

[illegible]

=> d [illegible] 1-[illegible]

L[illegible] ANSWER 1 OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: An approach for reducing unwanted oligomerisation of DsRed fusion proteins.
AUTHOR: Gavin Paul; Devenish Rodney J; Prescott Mark
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Monash University, Clayton, Vic., Australia.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, [illegible]
Journal code: [illegible]. ISSN: [illegible].
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: 20021107
Last Updated on STN: 2[illegible]30116
Entered Medline: 20030115

AB **Oligomerisation** of the red **fluorescent protein**, DsRed, can interfere with the localisation and function of proteins to which it is fused. We demonstrate an approach that may help to reduce significantly the impact of oligomerisation on the biology of the protein fusion partner. Growth of yeast (Saccharomyces cerevisiae) cells expressing ATP synthase containing subunit gamma-DsRed fusion was compromised relative to control cells. Furthermore, ATP synthase was found to exist as oligomeric structures when isolated under conditions where monomers would normally be present. The compromised growth phenotype was partially reversed and the oligomerisation of the ATP synthase [illegible] when a non-fluorescent variant of DsRed not fused to another protein was targeted to the mitochondrion in addition to the gamma-DsRed fusion protein. This strategy may also be applicable to the prevention of unwanted interactions between fusion proteins that contain the normally **dimeric fluorescent proteins** [illegible] Renilla **GFP**.

L[illegible] ANSWER 2 OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Far-red fluorescent tag for protein labelling.
AUTHOR: Fradkov Arkady F; Verkhusha Vladislav V; Staroverov Dmitry B; Bulina Maria E; Yanushevich Yurii G; Martynov Vladimir I; Lukyanov Sergey; Lukyanov Konstantin A
CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry

[illegible]

RAS, Miklukho-Maklaya [illegible], Moscow [illegible], Russia.

SOURCE: BIOCHEMICAL JOURNAL, [illegible]
Journal code: [illegible]. ISSN: [illegible].

PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB Practical applications of **green** fluorescent protein ('**GFP**')-like fluorescent proteins ('FPs') from species of the class Anthozoa (sea anemones, corals and sea pens) are strongly restricted due to their **oligomeric** nature. Here we suggest a strategy to overcome this problem by the use of two covalently linked identical red FPs as **non-oligomerizing** fusion tags. We have applied this approach to the **dimeric** far-red **fluorescent protein** [illegible] and have demonstrated applicability of the tandem tag in the in vivo labelling of fine cytoskeletal structures and tiny [illegible]. In addition, a possibility of effective fluorescence resonance energy transfer ('FRET') between enhanced yellow FP mutant ('EYFP') and tandem [illegible] was demonstrated in a protease assay.

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Intracellular localization of Herpes simplex virus type 1 thymidine kinase fused to different fluorescent proteins depends on choice of fluorescent tag.
AUTHOR: Sóling Ariane; Simm Andreas; Rainov Nikolai
CORPORATE SOURCE: Molecular Neurooncology Laboratory, Department of Neurosurgery, Martin-Luther-University Halle-Wittenberg, Heinrich-Lamertw-Strasse 1, [illegible], Halle, Germany.. ariane.soling@medizin.uni-halle.de
SOURCE: FEBS LETTERS, (2002 Sep 11) [illegible] (1-3) 153-[illegible].
Journal code: [illegible]. ISSN: [illegible].
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: 20020911
Last Updated on STN: [illegible]1213
Entered Medline: [illegible]

AB Gene therapy employing the suicide gene prodrug activating system Herpes simplex virus type 1 thymidine kinase (HSV-TK)/ganciclovir (GCV) is effective in killing malignant tumor cells. Labeling of the HSV-TK enzyme with fluorescent proteins makes possible the non-invasive imaging of transduction efficiency, enzyme localization and activity in cell culture and in animal models of human cancers. Here we report the expression of HSV-TK fused with different fluorescent proteins (EGFP, DsRed1, DsRed2, [illegible]) and show that intracellular localization of the fusion products depends on the nature of the fluorescent tag despite the presence of several nuclear targeting signals within the enzyme itself. Coexpression of [illegible] HSV-TK fusion proteins with TK-EGFP or untagged HSV-TK allowed these proteins to enter the nucleus by inhibiting formation of red **fluorescent protein oligomers**. As enzyme localization may influence HSV-TK activity, this observation is of

[illegible]

[illegible] Therapy [illegible]

L[illegible] ANSWER [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Measurement of changes in fluorescence resonance energy transfer between gonadotropin-releasing hormone receptors in response to agonists.
AUTHOR: [illegible] Anna; [illegible] Michael
CORPORATE SOURCE: [illegible] Primate Research Center and [illegible] Health and Science University, Beaverton [illegible], USA.
SOURCE: METHODS, [illegible] Aug [illegible]
Journal code: 9426[illegible] ISSN: 1046-2023.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB **Oligomerization** of membrane-bound G-protein-coupled receptors has recently emerged as an important step in cellular signaling. Fluorescence resonance energy transfer (FRET) has emerged as a potential method for demonstrating in vivo protein-protein interactions and receptor dimerization. We have used chimeras of gonadotropin-releasing hormone (GnRH) receptors and various **fluorescent proteins** to investigate receptor **dimerization** in relation to receptor activation. Two pairs of FRET-compatible fluorescent proteins were used: sapphire with topaz, and enhanced **green** fluorescent protein (eGFP) with dsRed. Changes in the ratio between acceptor and donor fluorescence were measured after addition of buserelin, a GnRH agonist, and antide, a GnRH antagonist. For both pairs of fluorescent proteins, an increase in the ratio of acceptor to donor intensities was observed immediately after addition of buserelin as would be predicted if FRET occurred due to the microaggregation of receptors conjugated with different fluorescent proteins. No change in FRET was observed in time for cells in medium or after addition of antide. The increase in FRET signal was not uniform throughout the cell.

L[illegible] ANSWER [illegible] OF 24 MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Use of **fluorescence** resonance energy transfer to analyze **oligomerization** of G-**protein**-coupled receptors expressed in yeast.
AUTHOR: [illegible] Mark C; Blumer Kendall J
CORPORATE SOURCE: Department of Cell Biology and Physiology, [illegible], Washington University School of Medicine, [illegible] South Euclid Avenue, St. Louis, MO [illegible], USA.
SOURCE: METHODS, (2002 Aug) [illegible] 324-32.
Journal code: 9426[illegible] ISSN: 1046-2023.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]

[illegible]

Entered Medline: [illegible]

AB **Oligomerization** [illegible] dimerization [illegible] G-protein-coupled receptors (GPCRs) has emerged as an important theme in signal transduction. This concept has recently gained widespread interest due to the application of [illegible] and quantitative biophysical techniques such as fluorescence resonance energy transfer (FRET), which have shown unequivocally that several types of GPCR can form dimers or **oligomers** in living cells. Current challenges are to determine which GPCRs can self-associate and/or interact with other GPCRs, to define the molecular principles that govern these specific interactions, and to determine which aspects of GPCR function require **oligomerization**. Although these questions must ultimately must be addressed by using GPCRs expressed endogenously in their native cell types, analysis of GPCR **oligomerization** in heterologous expression systems will be useful to survey which GPCRs can interact, to conduct structure-function studies, and to identify peptides or small molecules that disrupt GPCR **oligomerization** and function. Here, we describe methods employing scanning fluorimetry to detect FRET between GPCRs tagged with enhanced cyan and yellow fluorescent proteins (CFP and YFP) in living yeast cells. This approach provides a powerful means to analyze **oligomerization** of a variety of GPCRs that can be expressed in yeast, such as adrenergic, adenosine, [illegible], muscarinic acetylcholine, [illegible] protein, [illegible], and somatostatin receptors.

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: **Oligomerization** of DsRed is required for the generation of a functional red fluorescent chromophore.
AUTHOR: [illegible]etti Andrea; Subramaniam Vinod; Jovin Thomas M; Alberti Saverio
CORPORATE SOURCE: Laboratory of Experimental Oncology, Department of Cell Biology and Oncology, Istituto di Ricerche Farmacologiche Mario Negri-Consorzio Mario Negri Sud, 66030, Chieti, Santa Maria Imbaro, Italy.
SOURCE: FEBS LETTERS, (2002 Aug 14) 525 (1-3) 13-9. Journal code: 155157. ISSN: 0014-579[illegible].
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]09
ENTRY DATE: Entered STN: 2[illegible]
Last Updated on STN: 20[illegible]21218
Entered Medline: [illegible]

AB The [illegible] red fluorescent protein (DsRed) absorbs and emits light at much higher wavelengths than the structurally homologous **green** fluorescent protein, raising questions about the properties of its chromophore. We have analyzed the relationship between the aggregation state and fluorescence of native, [illegible]-histidine-tagged, or maltose-binding protein-fused DsRed. In all cases, newly synthesized DsRed molecules were largely monomeric and devoid of covalently closed chromophores. Maturation in vitro induces the expression of red **fluorescent** chromophores, but only in **oligomeric** forms of the **protein**, whereas monomers are essentially devoid of fluorescence. NaOH-denatured samples demonstrated a generalized breakdown of the DsRed **oligomers** to monomers, which refolded after neutralization into weakly **green** fluorescent and still monomeric species. Red fluorescent chromophores were regenerated only upon **oligomerization**. These findings demonstrate that 'red'

Search completed by David Schreiber [illegible]

[illegible]

[illegible] oligomers, [illegible] [illegible] oligomerization [illegible]

[illegible]

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Oligomerization [illegible] green fluorescent protein in the secretory pathway [illegible] cells.
AUTHOR: [illegible]
CORPORATE SOURCE: [illegible], University [illegible], [illegible] Street, [illegible], KY [illegible], U.S.A.
CONTRACT NUMBER: [illegible]
SOURCE: BIOCHEMICAL JOURNAL, [illegible]. Journal code: [illegible]. ISSN: [illegible].
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB Green [illegible] GFP [illegible] is used extensively as a reporter protein to monitor cellular processes, including intracellular protein trafficking and secretion. In general, this approach depends on GFP acting as a passive reporter protein. However, it was recently noted that GFP oligomerizes in the secretory pathway of endocrine cells. To characterize this oligomerization and its potential role in GFP transport, cytosolic and secretory forms of enhanced GFP (EGFP) were expressed in GH4C1 and AtT-20 endocrine cells. Biochemical analysis showed that cytosolic EGFP existed as a [illegible], whereas secretory forms of EGFP formed disulphide-linked oligomers. EGFP contains two cysteine residues (Cys[illegible] and Cys[illegible]), which could play a role in this oligomerization. Site-directed mutagenesis of Cys[illegible] and Cys[illegible] showed that both cysteine residues were involved in disulphide interactions. Substitution of either cysteine residue resulted in a reduction or loss of oligomers, although dimers of the secretory form of EGFP remained. Mutation of these residues did not adversely affect the fluorescence of EGFP. EGFP oligomers were stored in secretory granules and secreted by the regulated secretory pathway in endocrine AtT-20 cells. Similarly, the [illegible] forms of EGFP were still secreted via the regulated secretory pathway, indicating that the higher-order oligomers were not necessary for sorting in AtT-20 cells. These results suggest that the oligomerization of EGFP must be considered when the protein is used as a reporter molecule in the secretory pathway.

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Oligomerization [illegible] fluorescent protein-[illegible]

TSHR reveals post-translational complexes.

AUTHOR: Latif R; Graves P; Davies T F

CORPORATE SOURCE: Division of Endocrinology, Diabetes and Bone Diseases, Mount Sinai School of Medicine, New York, New York [illegible], USA.. rauf.latif@mssm.edu

CONTRACT NUMBER: [illegible] (NIDDK)
[illegible] (NIDDK)
[illegible] (NIDDK)
[illegible] (NIDDK)

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, [illegible]
[illegible].
Journal code: [illegible]. ISSN: [illegible].

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: [illegible]

ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB To examine thyrotropin (TSH) receptor (TSHR) interactions we fused the human TSH receptor (TSHR) carboxyl terminus to **green** fluorescent protein (**GFP**) and the corresponding chimeric cDNA was expressed in Chinese hamster ovary cells. Fluorescent TSH receptors on the plasma membrane were functional as assessed by TSH-induced cAMP generation. The binding of TSH, as well as TSHR autoantibodies, induced time- and dose-dependent receptor capping. Fluorescence resonance energy transfer between receptors differentially tagged with **GFP** variants (CFP and YFP) provided evidence for the close proximity of individual receptor molecules. This was consistent with previous studies demonstrating the presence of TSHR dimers and oligomers in thyroid tissue. Co-immunoprecipitation of **GFP**-tagged and Myc-tagged receptor complexes was performed using doubly transfected cells with Myc antibody. Western blotting of the immunoprecipitated complex revealed the absence of noncleaved TSH holoreceptors. This further suggested that cleavage of the holoreceptor into its two-subunit structure, comprising disulfide-linked TSHR-alpha and TSHR-beta subunits, was required for the formation of TSHR dimers and higher order complexes.

L[illegible] ANSWER [illegible] OF [illegible] MEDLINE

ACCESSION NUMBER: [illegible]1219272 MEDLINE

DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]

TITLE: Self-assembly and binding of a sorting nexin to sorting endosomes.

AUTHOR: Kurten R C; Eddington A D; Chowdhury P; Smith R D; Davidson A D; Shank B B

CORPORATE SOURCE: Department of Physiology, University of Arkansas for Medical Sciences, Little Rock, Arkansas [illegible], USA.. KurtenRichardC@exchange.uams.edu

SOURCE: JOURNAL OF CELL SCIENCE, (2001 May) 114 (Pt [illegible]) [illegible].
Journal code: [illegible]. ISSN: [illegible].

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: [illegible]

ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

[illegible]

AB [illegible]
[illegible] processing system in sorting endosomes.
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible] Using
green fluorescent protein fusions, endocytic compartment markers
and [illegible] recovery after photobleaching, we found that there is an
equilibrium between free cytoplasmic and early sorting endosome-bound
[illegible] green fluorescent protein-SNX1. Fluorescence resonance
energy transfer indicated that spectral variants of green
fluorescent protein-SNX1 were oligomeric in
vivo. In cell extracts, these green fluorescent
protein-SNX1 oligomers [illegible]
[illegible] green fluorescent protein-SNX1. Using video
microscopy, we observed small vesicles [illegible]
[illegible] green fluorescent protein-SNX1 [illegible] endosomes, which are
[illegible] with their role as sorting endosomes.
http://www.[illegible].html

L[illegible] ANSWER [illegible] OF 14 MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral.
AUTHOR: Baird G S; Zacharias D A; Tsien R Y
CORPORATE SOURCE: Department of Pharmacology, University of California, San Diego, La Jolla, CA 92093, USA.
CONTRACT NUMBER: [illegible] (NINDS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2000 Oct 24) 97 (22) 11984-9. Journal code: [illegible]. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]11
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB DsRed is a recently cloned 28-kDa fluorescent protein responsible for the red coloration around the oral disk of a coral of the Discosoma genus. DsRed has attracted tremendous interest as a potential expression tracer and fusion partner that would be complementary to the homologous green fluorescent protein from Aequorea, but very little is known of the biochemistry of DsRed. We now show that DsRed has a much higher extinction coefficient and quantum yield than previously reported, plus excellent resistance to pH extremes and photobleaching. In addition, its [illegible]-nm emission maximum can be further shifted to [illegible] nm by mutation of Lys-[illegible] to Met. However, DsRed has major drawbacks, such as strong oligomerization and slow maturation. Analytical ultracentrifugation proves DsRed to be an obligate tetramer in vitro, and

[illegible] assay [illegible] **oligomerization** in live cells. [illegible] **green** [illegible] in vitro [illegible] in vivo, and [illegible]. Many [illegible] applications of DsRed will require suppression of the tetramerization and acceleration of the maturation.

L4 ANSWER [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: Determination of the frequency of retroviral recombination between two identical sequences within a provirus.
AUTHOR: [illegible]; Zhang J
CORPORATE SOURCE: Department of Microbiology and Immunology and Markey Cancer Center, University of Kentucky, Lexington, Kentucky [illegible], USA.
CONTRACT NUMBER: [illegible]
SOURCE: JOURNAL OF VIROLOGY, [illegible] Aug [illegible].
Journal code: [illegible]. ISSN: [illegible].
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB Retroviruses use **RNA** as their genetic material within viral particles and **DNA** proviruses as their genetic material within cells. The rate of recombination during reverse transcription between two identical sequences within the same **RNA** molecule is very high. In this study, we have developed a sensitive system to study recombination occurring within the proviral sequence. This system includes a murine Moloney leukemia virus vector which contains a neomycin resistance gene (neo) and two mutated **green fluorescent protein** genes (**gfp**) in **tandem** positions. The 3' end of the first **gfp** and the 5' end of the second **gfp** gene are both mutated, so that neither of these two **gfp** genes is functional. However, if recombination occurs between the two **gfp** genes it will create a functional **gfp** protein. Cells containing such a functional recombinant **gfp** appear **green** under fluorescence microscopy. The rate of recombination between the two **gfp** sequences during a single round of replication is as high as [illegible]. **Green** cells appear during proliferation of a clonal [illegible] population and allow a small portion of these recombinations between sequences of proviral **DNA** to be detected. The frequency of recombination at the proviral **DNA** level is about [illegible] events per cell division, which is very low compared with the frequency of recombination ([illegible]) caused by reverse transcriptase and/or **RNA** polymerase II.

L4 ANSWER [illegible] MEDLINE
ACCESSION NUMBER: [illegible] MEDLINE
DOCUMENT NUMBER: [illegible] PubMed ID: [illegible]
TITLE: [illegible] of N-terminal alpha-helix formation in the [illegible] and intracellular targeting of [illegible] transferase.
AUTHOR: [illegible]
CORPORATE SOURCE: MRC Laboratory for Molecular Cell Biology and the [illegible]

[illegible]

Department of Biology, University College London, Gower Street, London WC1E 6BT, United Kingdom.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, [illegible] 274 [illegible]
[illegible].
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB The [illegible] peroxisome-to-mitochondrion mistargeting of alanine:glyoxylate aminotransferase (AGT) in the hereditary disease primary hyperoxaluria type 1 is caused by the combined presence of a common Pro11 --> Leu polymorphism and a disease-specific Gly170 --> Arg mutation. The Pro11 --> Leu replacement generates a functionally weak N-terminal mitochondrial targeting sequence (MTS), the efficiency of which is increased by the additional presence of the Gly170 --> Arg replacement. AGT dimerization is inhibited in the combined presence of both replacements but not when each is present separately. In this paper we have attempted to identify the structural determinants of AGT dimerization and mitochondrial mistargeting. Unlike most MTSs, the polymorphic MTS of AGT has little tendency to adopt an alpha-helical conformation in vitro. Nevertheless, it is able to target efficiently a monomeric **green fluorescent** (**GFP**) fusion **protein**, but not **dimeric** AGT, to mitochondria in transfected COS-1 cells. Increasing the propensity of this MTS to fold into an alpha-helix, by making a double Pro11 --> Leu + Pro10 --> Leu replacement, enabled it to target both **GFP** and AGT efficiently to mitochondria. The double Pro11 --> Leu + Pro10 --> Leu replacement retarded AGT dimerization in vitro as did the disease-causing double Pro11 --> Leu + Gly170 --> Arg replacement. These data suggest that N-terminal alpha-helix formation is more important for maintaining AGT in a conformation (i.e. monomeric) compatible with mitochondrial import than it is for the provision of mitochondrial targeting information. The parallel effects of the Pro10 --> Leu and Gly170 --> Arg replacements on the dimerization and intracellular targeting of polymorphic AGT (containing the Pro11 --> Leu replacement) raise the possibility that they might achieve their effects by the same mechanism.

L[illegible] ANSWER 1[illegible] OF 24 MEDLINE
ACCESSION NUMBER: 97446032 MEDLINE
DOCUMENT NUMBER: 97446032 PubMed ID: 92[illegible]
TITLE: **Oligomerization** of expanded-polyglutamine domain **fluorescent** fusion **proteins** in cultured mammalian cells.
AUTHOR: Onodera O; Burke J R; Miller S E; Hester S; Tsuji S; Roses A D; Strittmatter W J
CORPORATE SOURCE: Department of Medicine (Neurology), Duke University Medical Center, Durham, North Carolina 27710, USA.
CONTRACT NUMBER: [illegible]
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1997 Sep 18) 238 (2) 599-605.
Journal code: [illegible]. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

Harrison, [illegible]

FILE SEGMENT: Priority Journals
ENTRY MONTH: [illegible]
ENTRY DATE: Entered STN: [illegible]
Last Updated on STN: [illegible]
Entered Medline: [illegible]

AB Six inherited neurologic diseases, including Huntington's disease, result from the expansion of a CAG domain of the disease genes, producing a domain of more than 40 glutamines in the expressed protein. The mechanism by which expansion of this polyglutamine domain causes disease is unknown. In order to study the potential **oligomerization** of polyglutamine-domain proteins in mammalian cells, to study **oligomerization** of polyglutamine proteins and to identify heterologous protein interactions, varying length polyglutamine-**green** fluorescent protein fusion proteins were expressed in cultured COS-7 cells. The 19- and 35-glutamine fusion proteins (non-pathologic length) distributed diffusely throughout the cytoplasm. In contrast, 56- and 80-glutamine fusion proteins (pathologic length) formed fibrillar arrays resembling those previously observed in neurons in Huntington's disease and in a transgenic mouse model. These aggregates were intranuclear and intracytoplasmic. Intracytoplasmic aggregates were surrounded by collapsed intermediate filaments. The intermediate filament protein vimentin co-immunoisolated with expanded polyglutamine fusion proteins. This cellular model will expedite investigations into **oligomerization** of polyglutamine proteins and their interactions with other proteins.
Copyright [illegible] Academic Press.

L[illegible] ANSWER 14 OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: Systemically delivered antisense oligomers upregulate gene expression in mouse tissues
AUTHOR(S): Sazani, Peter; Gemignani, Federica; Kang, Shin-Hong; Maier, Martin A.; Manoharan, Muthiah; Persmark, Magnus; Bortner, Donna; Kole, Ryszard
CORPORATE SOURCE: Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, 27599, USA
SOURCE: Nature Biotechnology (20(12)), 2002, 12[illegible]
CODEN: NABIF9; ISSN: 1087-0156
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Systemically injected 2'-O-methoxyethyl (2'-O-MOE)-phosphorothioate and PNA-4K oligomers (peptide **nucleic** acid with four lysines linked at the C terminus) exhibited sequence-specific antisense activity in a no. of mouse organs. Morpholino oligomers were less effective, whereas PNA oligomers with only one lysine (PNA-1K) were completely inactive. The latter result indicates that the four-lysine tail is essential for the antisense activity of PNA oligomers in vivo. These results were obtained in a transgenic mouse model designed as a pos. readout test for activity, delivery, and distribution of antisense oligomers. In this model, the expressed gene (EGFP-654) encoding enhanced **green** fluorescence protein (EGFP) is interrupted by an aberrantly spliced mutated intron of the human β-globin gene. Aberrant splicing of this intron prevented expression of EGFP-654 in all tissues, whereas in tissues and organs that took up splice site-targeted antisense oligomers, correct splicing was restored and EGFP-654 expression upregulated. The sequence-specific ability of PNA-4K and the 2'-O-MOE oligomers to upregulate EGFP-654 provides the first evidence that systemically delivered, chem. modified

[illegible]

[illegible] effect and expression by sequence-specific [illegible] antiviral activity, validating their application as potential therapeutics.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: [illegible] **dimeric fluorescent protein** [illegible]
INVENTOR(S): Davis, Ronald W.; Vaillancourt, Peter
PATENT ASSIGNEE(S): Stratagene, USA
SOURCE: PCT Int. Appl., [illegible] pp.
CODEN: [illegible]
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO [illegible]	A[illegible]	[illegible]	WO [illegible]	[illegible]

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, [illegible]
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002230920	A5	20020624	AU 2002-30920	20011213

PRIORITY APPLN. INFO.: US 2000-256121P P 20001215; WO [illegible] W [illegible]

AB The invention relates to proteins or polypeptides that comprise intramol. **dimers** of **fluorescent protein** monomers. More specifically, the invention relates to recombinant polypeptides comprising a monomer of a fluorescent polypeptide, a linker peptide, and a second monomer of that fluorescent polypeptide, where the monomers form an intramol. dimer. The invention also relates to **nucleic** acids encoding Intramol. **Dimer Fluorescent Proteins** (IDFPs) and vectors comprising such a **nucleic** acids. The invention further relates to methods of making IDFPs and methods of using them. IDFPs are useful in any application suited for fluorescent proteins and are particularly useful in applications in which more than one **fluorescent protein** sharing complementary **dimerization** interfaces is present in the same mixt. or is expressed in the same cell, because IDFPs do not form heterodimers.

L4 ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT [illegible] ACS
ACCESSION NUMBER: [illegible] HCAPLUS
Correction of: [illegible]
DOCUMENT NUMBER: [illegible]
Correction of: [illegible]
TITLE: Proteins of hepatitis C virus showing specific interactions and their use in prevention and treatment of infection

[illegible]

```
INVENTOR(S):             Legrain, Pierre; Whiteside, Simon; Wojcik, Jerome
PATENT ASSIGNEE(S):      Hybrigenics S.A., Fr.
SOURCE:                  Eur. Pat. Appl., [illegible] pp.
                         CODEN: EPXXDW
DOCUMENT TYPE:           Patent
LANGUAGE:                English
FAMILY ACC. NUM. COUNT:  1
PATENT INFORMATION:

     PATENT NO.          KIND  DATE          APPLICATION NO.  DATE
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     EP [illegible]      A1    [illegible]   EP [illegible]   [illegible]
         R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, LT, LV, FI, RO
     [illegible]         A1    [illegible]   [illegible]      [illegible]
     [illegible]         A1    [illegible]
         W: [illegible]
         RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE, TR
     [illegible]         A1    [illegible]   [illegible]      [illegible]
PRIORITY APPLN. INFO.:                  EP [illegible]   A [illegible]
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AB The present invention relates to **nucleic** acids encoding SID (Selected Interacting Domain) polypeptides: minimal fragments of hepatitis C virus proteins responsible for specific interactions with proteins of the virus. These peptides and their interactions may be targets for prevention and treatment of infection of hepatitis C virus. The invention also includes vectors comprising a **nucleic** acid encoding a SID polypeptide as well as host cells transformed with such vectors. The invention is also directed to yeast or bacterial two-hybrid methods which make use of the **nucleic** acids encoding a SID polypeptide selected from a pathogenic strain of the hepatitis C virus as well as to methods for selecting mols. which inhibit the binding between a SID polypeptide and a polypeptide which specifically binds thereto. Protein-protein interactions of SID polypeptides may be detected by **covalent** or non-**covalent** attachment of **fluorescent proteins**, labeled antibodies or enzymes with catalytic activity to the SID polypeptide (marker protein) and subsequently contacting the marker protein with a plurality of other proteins like SID polypeptides. The SID polypeptides may also be covalently linked to a spacer, which may also be covalently bound to a substrate to immobilize the SID polypeptides. For example, the SID polypeptide may be attached to biotin when the substrate is streptavidin. Changes in optical properties of the substrate, following binding by SID polypeptides are detected. SID polypeptides can be used in vaccines for prevention and treatment of hepatitis C virus in animals or humans.

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L24  ANSWER [illegible] OF 24  HCAPLUS  COPYRIGHT [illegible] ACS
ACCESSION NUMBER:        [illegible] HCAPLUS
DOCUMENT NUMBER:         [illegible]
TITLE:                   Single molecule imaging of green fluorescent
                         proteins in living cells: E-cadherin forms
                         oligomers on the free cell surface
AUTHOR(S):               Iino, Ryota; Koyama, Ikuko; Kusumi, Akihiro
CORPORATE SOURCE:        Kusumi Membrane Organizer Project, Exploratory
                         Research for Advanced Technology Organization, Japan
                         Science and Technology Corporation, Nagoya, [illegible],
                         Japan
SOURCE:                  Biophysical Journal [illegible]
                         CODEN: BIOJAU; ISSN: [illegible]
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[illegible]

PUBLISHER: Biophysical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Single green fluorescent protein (GFP) molecules were successfully imaged for the first time in living cells. GFP linked to the cytoplasmic carboxyl terminus of E-cadherin (E-cad-GFP) was expressed in mouse fibroblast L cells, and observed using an objective-type total internal reflection fluorescence microscope. Based on the fluorescence intensity of individual fluorescent spots, the majority of E-cad-GFP molecules in the free cell surface were found to form oligomers of various sizes, many of them greater than dimers, indicating that oligomerization of E-cadherin takes place prior to its assembly at cell-cell adhesion sites. The translational diffusion coeff. of E-cad-GFP is reduced by a factor of [illegible] upon oligomerization. Because such large decreases in translational mobility cannot be explained solely by increases in radius upon oligomerization, an oligomerization-induced trapping model is proposed in which, when oligomers are formed, they are trapped in place due to greatly enhanced tethering and corralling effects of the membrane skeleton on oligomers compared with monomers. The presence of many oligomers greater than dimers on the free surface suggests that these greater oligomers are the basic building blocks for the two-dimensional cell adhesion structures (adherens junctions).
REFERENCE COUNT: [illegible] THERE ARE [illegible] CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: Collective effects in individual oligomers of the red fluorescent coral protein DsRed
AUTHOR(S): Cotlet, M.; Hofkens, J.; Kohn, F.; Michiels, J.; Dirix, G.; Van Guyse, M.; Vanderleyden, J.; De Schryver, F. C.
CORPORATE SOURCE: Department of Chemistry, Katholieke Universiteit Leuven, Heverlee, [illegible], Belg.
SOURCE: Chemical Physics Letters (2001), [illegible], 415-42[illegible]
CODEN: CHPLBC; ISSN: 0009-2614
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We report on single-mol. expts. that were performed on two biol. systems, the recently cloned red fluorescent protein of a coral of the Discosoma genus (DsRed) and the enhanced green fluorescent protein, both of which were immobilized in polyvinyl alc. Fluorescence intensity trajectories were recorded and compared to those of a ref. compd., the rigid dye hexaphenylbenzene perylenemonoimide. Evidence was found for the presence of DsRed as an oligomer even at sub-nanomolar concns. Both proteins show triplet blinking in the millisecond time range. Collective effects could be obsd. for the DsRed oligomers.
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L[illegible] ANSWER [illegible] OF [illegible] HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: [illegible] HCAPLUS
DOCUMENT NUMBER: [illegible]
TITLE: Oligomerization of the human [illegible]

transporter and of the rat GABA transporter 1 visualized by fluorescence resonance energy transfer microscopy in living cells

AUTHOR(S): Schmid, Johannes A.; Scholze, Petra; Kudlacek, Oliver; Freissmuth, Michael; Singer, Ernst A.; Sitte, Harald H.

CORPORATE SOURCE: Department of Vascular Biology and Thrombosis Research, University of Vienna Medical School, Vienna, A-[illegible], Austria

SOURCE: Journal of Biological Chemistry ([illegible]), [illegible], [illegible]

CODEN: JBCHA3; ISSN: [illegible]

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Recent biochem. studies indicate that the serotonin transporter can form **oligomers**. We investigated whether the human serotonin transporter (hSERT) can be visualized as an **oligomer** in the plasma membrane of intact cells. For this purpose, we generated fusion proteins of hSERT and spectral variants of the **green** fluorescent protein (cyan and yellow fluorescent proteins, CFP and YFP, resp.). When expressed in human embryonic kidney 293 cells, the resulting fusion proteins (CFP-hSERT and YFP-hSERT) were efficiently inserted into the plasma membrane and were functionally indistinguishable from wild-type hSERT. **Oligomers** were visualized by fluorescence resonance energy transfer microscopy in living cells using two complementary methods, i.e. ratio imaging and acceptor photobleaching. Interestingly, **oligomerization** was not confined to hSERT; fluorescence resonance energy transfer was also found between CFP- and YFP-tagged rat .gamma.-aminobutyric acid transporter. The bulk of serotonin transporters was recovered as high mol. wt. complexes upon gel filtration in detergent soln. In contrast, the monomers of CFP-hSERT and YFP-hSERT were essentially undetectable. This indicates that the homo-**oligomeric** form is the favored state of hSERT in living cells, which is not significantly affected by coincubation with transporter substrates or blockers. Based on our observations, we conclude that constitutive **oligomer** formation might be a general property of Na^+/Cl^--dependent neurotransmitter transporters.

REFERENCE COUNT: 2[illegible] THERE ARE 2[illegible] CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ACCESSION NUMBER: 1998:[illegible] HCAPLUS

DOCUMENT NUMBER: 12[illegible]

TITLE: PRIM: proximity imaging of **green** fluorescent protein-tagged polypeptides

AUTHOR(S): De Angelis, Dino A.; Miesenbock, Gero; Zemelman, Boris V.; Rothman, James E.

CORPORATE SOURCE: Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1998), [illegible](21), 12312-12316

CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We report a serendipitous discovery that extends the impressive catalog of

[illegible] green [illegible]
GFP [illegible]. When two [illegible] are brought [illegible] proximity, changes in the relative intensities of green [illegible]. These spectral changes provide a sensitive [illegible] index of the extent of [illegible] that can be exploited [illegible]. Image [illegible] - oligomerization [illegible] clustering processes of GFP-tagged proteins in vivo. The method, which we term proximity imaging (PRIM), [illegible] energy transfer between [illegible] GFP [illegible], a powerful [illegible] proximity relationships between different proteins. However, [illegible] energy transfer [illegible] spectral [illegible], PRIM depends on direct contact between two GFP [illegible], which can lead to structural perturbations and [illegible] spectral changes within a module. Moreover, the precise spatial arrangement of the GFP [illegible] within a given dimer [illegible] the magnitude and direction of the spectral change. We have used PRIM to [illegible] FK[illegible]-induced dimerization of GFP fused to FK[illegible]-binding protein and clustering of glycosylphosphatidylinositol-anchored GFP [illegible].

REFERENCE COUNT: [illegible] THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 21 OF [illegible] BIOSIS COPYRIGHT [illegible] BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: [illegible] BIOSIS

DOCUMENT NUMBER: [illegible]

TITLE: AAV-Mediated Delivery of Rhodopsin [illegible] the Rhodopsin Knockout Mouse [illegible].

AUTHOR(S): [illegible], M. J. [illegible]; Liang, F. [illegible]; Maguire, A. M. [illegible]; Bennett, J. [illegible]

CORPORATE SOURCE: [illegible] Dept. of Ophthalmology, Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA, USA USA

SOURCE: ARVO Annual Meeting Abstract Search and Program Planner, (2002) Vol. 2002, pp. Abstract No. 460[illegible]. cd-rom. Meeting Info.: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May [illegible], 2002

DOCUMENT TYPE: Conference

LANGUAGE: English

AB Purpose: Rhodopsin knockout mice (rho-/-) lack outer segments, and photoreceptor degeneration is nearly complete by post-natal day [illegible] (Lem et al. 1999 PNAS, 96: 7[illegible]-741). This study aimed to deliver rhodopsin to these animals via an adeno-associated virus [illegible] (AAV2), and assess potential rescue effects. Methods: An AAV2, expressing the murine rhodopsin cDNA in tandem with the marker enhanced green fluorescent protein was prepared. Rho-/- mice (post-natal days [illegible]) subsequently received the virus subretinally (single [illegible]) and were maintained for [illegible] weeks. Contralateral eyes served as untreated controls. Five, [illegible] and 12 weeks post-injection, eyes were enucleated, fixed, cryoprotected and assessed histologically. Samples were analyzed for rhodopsin (primary antibody, rho4D2) and EGFP expression, as well as for evidence of photoreceptor rescue, using fluorescent microscopy. Results: Five weeks post-injection, EGFP expression [illegible] photoreceptor cell [illegible] and inner segments. Immunohistochemistry [illegible] rhodopsin labeling localized to the tips of the inner segments. A similar expression pattern was also evident at [illegible] weeks post-injection. At this time there also appeared [illegible] photoreceptors surrounding the site of injection; however, there was [illegible] evidence [illegible] outer segment [illegible], and the rescue effect was not

[illegible] and EGFP expression was [illegible] evident. [illegible] AAV-mediated [illegible] [illegible] phenotype. [illegible] AAV-mediated [illegible] different AAV [illegible] maximum [illegible].

L[illegible] ANSWER [illegible] BIOSIS COPYRIGHT [illegible] BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: [illegible]
DOCUMENT NUMBER: PREV[illegible]
TITLE: [illegible]-FRET microscopy in living cells to measure monomer-dimer transition of **GFP**-tagged proteins.
AUTHOR(S): [illegible], [illegible]; Tramier, M.; [illegible]; [illegible]; [illegible] R. H.; Nicolas, [illegible]; [illegible], K.; [illegible]-[illegible], M. [illegible]
CORPORATE SOURCE: [illegible] Institut Jacques Monod, [illegible], 2 Place Jussieu-Tour 43, [illegible], Paris [illegible] France
SOURCE: Biophysical Journal, [illegible], pp. [illegible] print.
[illegible]: [illegible].
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Fluorescence anisotropy decay microscopy was used to determine, in individual living cells, the spatial monomer-dimer distribution of proteins, as exemplified by herpes simplex virus thymidine kinase (TK) fused to **green** fluorescent protein (**GFP**). Accordingly, the fluorescence anisotropy dynamics of two fusion proteins (TK27GFP and TK366GFP) was recorded in the confocal mode by ultra-sensitive time-correlated single-photon counting. This provided a measurement of the rotational time of these proteins, which, by comparing with **GFP**, allowed the determination of their **oligomeric** state in both the cytoplasm and the nucleus. It also revealed energy homo-transfer within aggregates that TK366GFP progressively formed. Using a symmetric dimer model, structural parameters were estimated; the mutual orientation of the transition dipoles of the two **GFP** chromophores, calculated from the residual anisotropy, was 44.[illegible]+-1.[illegible]degree, and the upper intermolecular limit between the two fluorescent tags, calculated from the energy transfer rate, was [illegible] ANG. Acquisition of the fluorescence steady-state intensity, lifetime, and anisotropy decay in the same cells, at different times after transfection, indicated that TK366GFP was initially in a monomeric state and then formed dimers that grew into aggregates. Picosecond time-resolved fluorescence anisotropy microscopy opens a promising avenue for obtaining structural information on proteins in individual living cells, even when expression levels are very low.

L[illegible] ANSWER [illegible] BIOSIS COPYRIGHT [illegible] BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: [illegible] BIOSIS
DOCUMENT NUMBER: PREV199[illegible]
TITLE: A novel nuclear receptor heterodimerization pathway mediated by orphan receptors TR2 and TR4.
AUTHOR(S): Lee, Chin-Hac; Chinpaisal, Chatchai; Wei, Li-Na [illegible]
CORPORATE SOURCE: [illegible] Dep. Pharmacol., Univ. Minnesota, [illegible] Millard Hall, [illegible] Delaware St. S.E., MN [illegible] USA
SOURCE: Journal of Biological Chemistry, (Sept. [illegible], [illegible]) Vol. [illegible], No. [illegible], pp. [illegible].

ISSN: [illegible]
DOCUMENT TYPE: Article
LANGUAGE: English

AB [illegible] TR2 and TR4 [illegible] TR2 and TR4 [illegible] as well as [illegible] DNA [illegible]. [illegible] between TR2 and TR4 [illegible] assays, [illegible] assay, and [illegible] assay. [illegible] a green fluorescent protein. [illegible] dimerization [illegible], and [illegible] TR2 are [illegible]. In addition, [illegible] TR2-containing [illegible] alone. In the developing testis, TR2 and TR4 are [illegible] in the same testicular cell populations and exhibit a parallel pattern of expression along development. The preferential [illegible] between TR2 and TR4 and their [illegible] in specific [illegible] a physiological role of TR2/TR4 [illegible] in [illegible] development.

L[illegible] ANSWER [illegible] EMBASE COPYRIGHT [illegible] ELSEVIER SCI. B.V.
ACCESSION NUMBER: [illegible] EMBASE
TITLE: [illegible] and application of a GFP-[illegible] intracellular caspase assay for drug screening.
AUTHOR: [illegible]
CORPORATE SOURCE: [illegible] Molecular Pharmacology, [illegible] Corporation, [illegible] Torreyana Road, San Diego, CA [illegible], United States. [illegible]
SOURCE: Journal of Biomolecular Screening, [illegible]. Refs: 25
ISSN: [illegible] CODEN: JBISF3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: [illegible] Cancer
[illegible] Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Apoptosis is a crucial biological process, and activation of caspase endoproteases is essential for proper regulation and execution of apoptosis. Because caspases also appear to be central players in several pathological states, there is a practical need within the biopharmaceutical research community for facile, noninvasive cellular assays for the discovery of compounds that modulate caspase activity. Tandem [illegible] of green fluorescent protein (GFP) stably expressed within cells can serve as [illegible] sensor of protease activity. [illegible] this technology, we have developed a stable cellular system for the screening of agents that modulate activation of the caspase cascade. This assay technology allows for the real-time monitoring of apoptosis in situ, using conventional fluorescent plate reader detection. By applying this assay system to an actual compound screen, small-molecule inducers of cell apoptosis were [illegible] identified. Follow-up pharmacology confirmed that the [illegible] of primary hits using the intracellular GFP assay [illegible] that found using a conventional, cell lysis-based assay [illegible].

Search completed by David Schreiner [illegible]

Ham [illegible]

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FILE 'MEDLINE, [illegible], BIOSIS, EMBASE, [illegible], [illegible]' ENTERED AT [illegible] ON [illegible] MAY [illegible]

L14 [illegible] ALK[illegible] AND FLUORESCENT [illegible] PROTEIN#

Search completed by David Schreiber [illegible]-4292